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Breda, Inês Lousinha Ribeiro; Søborg, Ditte; Ramsay, Loren; Roslev, Peter

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Manganese removal processes during start-up of inoculated and non-inoculated drinking water biofilters

I. L. Breda, D. A. Søborg, L. Ramsay and P. Roslev

ABSTRACT

Manganese removal in drinking water biofilters is facilitated by biological and physico-chemical processes, but knowledge regarding the relative role of these mechanisms during start-up is very limited. The aim of this study was to identify the dominant process for manganese removal occurring during the start-up period of sand filters with and without inoculation by addition of matured sand collected from an operating groundwater-based waterworks. Inoculation with matured filter sand is frequently used to accelerate the start-up in virgin biofilters and to rapidly obtain compliant water quality. The non-inoculated filter took 41 days to comply with manganese quality criteria, whereas the inoculated filter with 20% matured sand showed removal from Day 1 and compliance from Day 25. By Day 48, the inoculated filter showed two times higher manganese removal rates and manganese oxides deposits. Using sodium azide as an inhibitor of microbial activity, it was found that manganese removal in the non-inoculated filter was dominated by biological processes, whereas physico-chemical processes were of more importance in the inoculated filter (Day 35, 39 and 48). 16S rDNA sequencing of the microbiota collected during filter maturation indicated a limited immediate effect of inoculation on the microbial community developed on the remaining filter material.

Key words | groundwater, inoculation, manganese removal processes, sodium azide, start-up

I. L. Breda (corresponding author)

P. Roslev

Department of Chemistry and Bioscience,
Aalborg University,
Fredrik Bajers Vej 7H, 9220 Aalborg,
Denmark
E-mail: ilb@bio.aau.dk

I. L. Breda

Group of Project and Development,
Skanderborg Forsyningsvirksomhed A/S,
Døjsøvej 1, 8660 Skanderborg,
Denmark

D. A. Søborg

L. Ramsay

Research Group for Energy and Environment,
VIA University College,
Chr. M. Østergaards Vej 4, 8700 Horsens,
Denmark

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INTRODUCTION

Biofilters are often used in production of drinking water from groundwater sources. However, a major disadvantage of biofiltration is the necessity of a start-up period to mature virgin filter media. When manganese is present in source water, the start-up period can last from weeks to more than a year (Tekerlekopoulou *et al.* 2013).

Proactive inoculation methods to accelerate the start-up of biofilters include the addition of a concentrated source of microorganisms and/or autocatalytic surfaces, e.g. backwash sludge (Štembal *et al.* 2004; Cai *et al.* 2015; Dangeti *et al.* 2017), matured filter sand (Zeng *et al.* 2010; Bruins 2016), mixed bacterial culture (Tekerlekopoulou & Vayenas

2008) or specific bacterial species (Qin *et al.* 2009; Bai *et al.* 2016; Li *et al.* 2016; McKee *et al.* 2016). The most common methods used by the drinking water industry are based on the addition of backwash sludge or matured filter sand (Štembal *et al.* 2004). Amendment with matured sand provides immediate removal, while potentially promoting the microbial growth on the virgin sand in the remaining filter.

Manganese removal in biofilters is based on physico-chemical and biological processes (Mouchet 1992). Recent studies suggest that after initial sorption, manganese removal by a non-coated virgin medium is initiated biologically, evolving to a predominantly physico-chemical removal process over time due to the development of an autocatalytic coating of manganese on the filter grains (Sahabi *et al.* 2009; Bruins 2016). This recent knowledge could be of help in reducing typically long start-up periods

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by creating conditions that are favorable for the growth of manganese oxidizing bacteria (MnOB) and other microorganisms involved in manganese oxidation.

Previous studies have investigated the importance of biological manganese oxidation in drinking water biofilters using various methods to inhibit biological removal (Vandeele *et al.* 1992; Gounot 1994; Olańczuk-Neyman & Bray 2000; Sahabi *et al.* 2009). However, further investigations are required to understand the contribution of different mechanisms of manganese removal during the start-up period.

The aim of this study was to identify the dominant process in manganese removal (physico-chemical and biological) during the start-up of a virgin sand pilot biofilter with and without inoculation by addition of matured sand. In addition, the aim of this study was to shed light on the effect of proactive inoculation by addition of a layer of matured filter sand on the microbial community developed in the adjacent virgin layers of the filter.

MATERIALS AND METHODS

Pilot scale set-up

Treated groundwater from the storage tank of a Danish drinking water treatment plant was used as source water (Fredensborg waterworks, Skanderborg, Denmark). The treated water contains a natural background of drinking water microorganisms as disinfection is not used at this and most other waterworks in Denmark. The unchlorinated treated water was continuously spiked with a concentrated solution of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Emsure ACS), using a diaphragm pump (Digital DDC, Grundfos), and distributed to two pressurized 0.3 m^3 filter tanks (Type NS20, Silhorko Euro-water). The filters were placed at the water treatment plant and operated at a temperature of 11°C (Table 1, Figure 1).

Each filter has a diameter of 30 cm and a 1 m layer of granular quartz sand. The non-inoculated filter was filled solely with virgin quartz sand (Dansk Kvarts Industri), and the inoculated filter with two intercalated layers of matured sand in the virgin sand (Figure 1, Table 2). The matured sand used for inoculation of the pilot filter was collected from the top layer of a second stage biofilter of Fredensborg waterworks, which had been removing manganese for the last 47 years. Throughout the experiment, no visual mixing

Table 1 | Source water quality

| Parameter | Unit | Average | Std. dev. |
|--------------------|------------------|---------|-----------|
| Manganese | mg/L | 0.281 | 0.018 |
| Iron | mg/L | 0.019 | 0.01 |
| Ammonium | mg/L | <0.02 | – |
| Nitrite | mg/L | <0.001 | – |
| Hydrogen carbonate | mg/L | 276 | 0.5 |
| pH | – | 7.95 | 0.05 |
| Oxygen | mg/L | 10.8 | 0.05 |
| Temperature | $^\circ\text{C}$ | 10.7 | 0.2 |
| NVOC ^a | mg/L | 1.0 | 0.1 |

^aNon-volatile organic carbon.

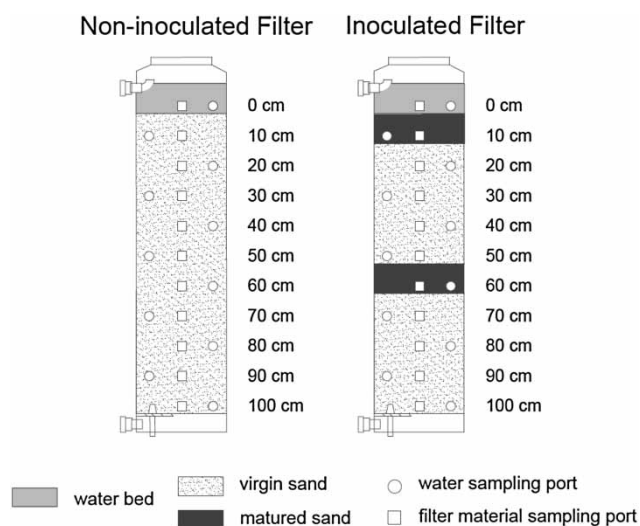


Figure 1 | Filter tanks and sampling depths for water and filter medium.

was observed between the two-filter media used in the inoculated filter (virgin and matured sand).

Before the start of operation, the filter tanks and the virgin sand medium were disinfected overnight with 2% H_2O_2 according to the manufacturer's standard procedures

Table 2 | Filter medium properties

| Parameter | Matured sand | Virgin sand |
|-------------------------|--------------|-------------|
| Grain size (mm, 10–90%) | 1.03–1.86 | 1.10–1.76 |
| Sphericity | 0.90 | 0.87 |
| Particle density (kg/L) | 2.50 | 2.60 |
| Porosity (%) | 42.6 | 40.6 |

which included successive backwashes to remove fines and excess H_2O_2 . Matured sand was added subsequently to ensure that its microbial community was not affected during disinfection. The filters were operated in downflow mode with a filtration rate of 5 m/h and an empty bed contact time of 12 min. No backwash was used after the start of operation.

Water and filter medium sampling

Inlet and outlet samples (10 mL) from the filters were manually collected each couple of days for a period of 72 days, filtered (0.22 μm) and analyzed immediately for total dissolved manganese. In addition, water samples were collected from both filters at 10 cm depth intervals (profile) once a week following the same procedure. Inlet water (4 L) was filtered (0.20 μm membrane filters, Advantec) and stored at -21°C for subsequent microbial diversity analysis.

Before the start of operations (Day 0) virgin sand and matured sand samples were collected to quantify the manganese coating the grains, to investigate the manganese removal rate and to analyze the microbial diversity. During operations (Day 35, 37 and 48) filter media samples were collected from both filters to quantify the manganese coating the grains (depth 10, 20 and 30 cm), to investigate the manganese removal rate (depth 10, 20 and 60 cm) and to analyze the microbial diversity (depth 10, 20, 30, 60 and 80). Sampling days during operations were selected with focus on the period in which the non-inoculated filter started to remove manganese. A previous investigation conducted by Breda et al. (2017) in column scale using the same source water and filter materials as in the present study indicated that manganese removal would start shortly after 30 days. Sampling depths were defined to represent the most active sections of the filters. One month after the end of the start-up period of the non-inoculated filter (Day 72) filter media from the top 10 cm of each filter were collected to investigate the manganese removal rate.

Manganese measurements in water and medium samples

Total dissolved manganese was measured according to the manufacturer's instructions (kit LCK304, lower level of

detection of 0.005 mg/L, DR3900 Spectrophotometer, Hach, Denmark).

The coating of filter medium samples (0.5 g in duplicates) was extracted in a 50 mL mixture of 4 M HCl and 2 g/L oxalic acid ($\text{C}_2\text{H}_2\text{O}_4$) as described by De Vet et al. (2009). The manganese concentration in the decanted acid solution was measured using inductively coupled plasma optical emission spectroscopy (ICP-OES) according to *Standard Methods* (American Public Health Association 1975).

Batch assay with and without NaN_3 for determining manganese removal rates

Filter medium samples (1 g) were placed in four serum bottles with 25 mL deionized water. Half of the bottles were incubated for 1 h with NaN_3 (25 mM, Merck KGaA) to inhibit microbial respiration (Tebo et al. 2005). All bottles were then spiked with $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Emsure ACS) to obtain an initial concentration of 0.3 mg/L. Water samples (1.5 mL) were collected each 5 min for 20 min, filtered (0.22 μm) and analyzed for total dissolved manganese (kit LCK304, Hach, Denmark using a Multiskan FC Microplate Photometer, Thermo Fisher Scientific). All bottles were continually mixed on an orbital shaker (150 rpm) at room temperature during the experiment. Control bottles without filter medium, with and without NaN_3 , were included to account for any precipitation or sorption of manganese to glass surfaces of the incubation bottles.

Column assay with and without NaN_3 for determining manganese removal rates

Filter medium samples (100 g in quadruplicate) from the top 10 cm of each filter were collected one month after the end of the start-up period (Day 72). Immediately after sampling, two replicates of the medium samples from each filter were pretreated by immersion in a 25 mM NaN_3 solution (Merck KGaA). An eight-column assay with four columns with NaN_3 and four columns without NaN_3 was constructed using the same filtration rate as the pilot filters. Source water consisted of treated water from the waterworks spiked with $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (Emsure ACS) to obtain an initial concentration of 0.3 mg Mn/L. To ensure a continuous inhibition of microbial respiration, 25 mM NaN_3 was added to the

source water feeding the columns with NaN_3 pretreatment. The manganese removal capacity of the filter columns was followed for 3 h. Water samples were collected each hour from the outlet of each column, filtered ($0.22\ \mu\text{m}$) and analyzed for total dissolved manganese as described above.

DNA extraction and 16S rDNA amplicon sequencing and library preparation

DNA was extracted from filter medium samples using the FastDNA spin kit for soil (MP Biomedicals) and from water samples using the PowerWater DNA Isolation Kit (MOBIO). A full description of the methodology used for quantitative polymerase chain reaction (qPCR) amplification, 16S rDNA amplicon sequencing and library preparation can be found in the supplementary material (available with the online version of this paper). Rarefaction curves for all individual samples were determined to ensure exhaustive sequencing of the diversity in the sample.

Bioinformatics and statistical analysis

Statistical analysis including the non-parametric Mann-Whitney U test was performed in R through Rstudio IDE (R Core Team 2017). A nominal p -value less than 0.05 was considered to be of statistical significance. Results from 16S rDNA amplicon sequencing were analyzed using the ampvis package v.2.0.0 (Albertsen et al. 2015). Multivariate statistics based on principal component analysis (PCA) were carried out to compare the bacterial communities in the different filter sand samples, using the amp_ordinate function with Hellinger transformed operational taxonomic unit (OTU) counts (Albertsen et al. 2015).

RESULTS AND DISCUSSION

Manganese removal in inoculated and non-inoculated filters during the start-up period

Manganese removal was not detected in the non-inoculated pilot filter (virgin sand) during the first 30 days of the start-up period with exception of initial adsorption at Day 0 (Figure 2). Compliance with the drinking water criterion of

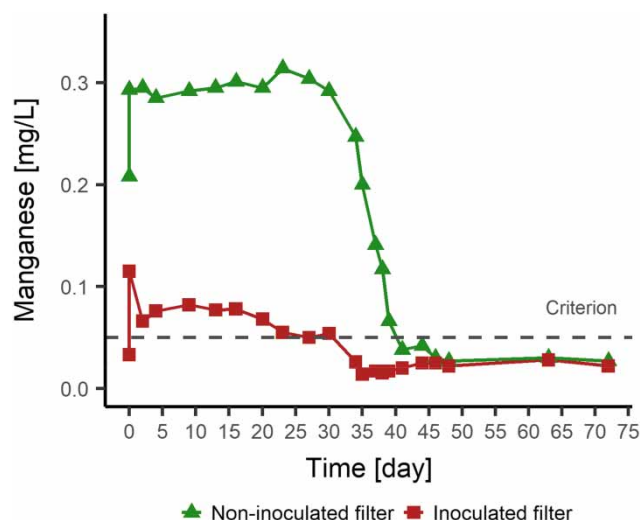


Figure 2 | Manganese concentration over time at the outlet of the pilot biofilters with and without inoculation.

0.05 mg/L was observed on Day 41 (Figure 2). Similar removal patterns by initially non-coated virgin filter media in columns treating solely manganese have been reported previously (Bruins 2016; Breda et al. 2017).

The inoculated pilot filter with 20% matured sand showed significant removal (approximately 65–80%) from Day 1. Compliance for the inoculated filter was achieved after 25 days of operation (Figure 2). After 48 days, manganese removal in the inoculated and non-inoculated filters was comparable (approximately 90% removal).

The investigation by Bruins (2016) showed no difference in manganese removal efficiency during the start-up period of a non-inoculated and an inoculated (7.5% matured manganese coated sand) full-scale filter using groundwater as source water. The absence of immediate manganese removal by the inoculated filter was assumed to be caused by the loss of the autocatalytic and biological activity of MnOx and MnOB , respectively coating the medium during storage in open air for several months. In contrast, results of this study indicate that inoculation with fresh matured manganese coated sand accelerates the onset of manganese removal and that the long start-up period for manganese can be substantially reduced by initially supplementing the filter vessel with approximately 20% fresh matured manganese coated sand.

The non-inoculated filter (Figure 3(a)) initially showed no detectable manganese removal (Day 6 and 20) followed

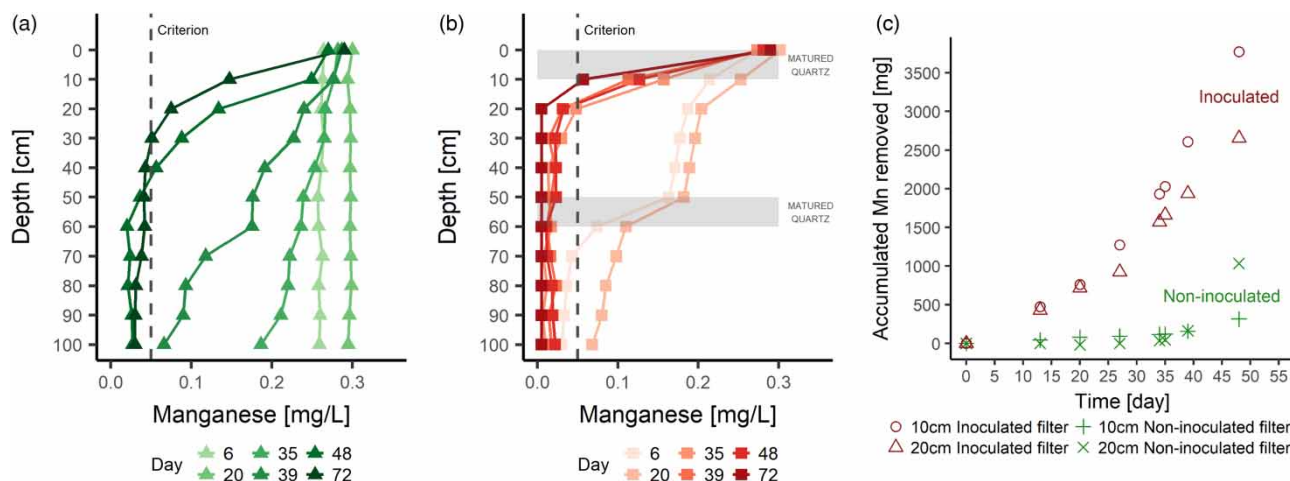


Figure 3 | Manganese concentration profile over time in (a) the non-inoculated filter and (b) the inoculated filter. (c) Total manganese removed by both non-inoculated and inoculated filter at depth 10 and 20 cm over time.

by slight manganese removal at all depths (Day 35 and 39). In contrast, the initial manganese concentration profile for the inoculated filter (Day 6, Figure 3(b)) shows a step pattern which clearly indicates the location of the mature sand layers (10 and 60 cm, Figure 1). At these depths, an especially high removal of manganese occurred. Slight manganese removal also occurred in the layers directly under the mature sand.

Manganese removal in the non-inoculated filter continued to increase throughout the investigation period (Figure 3(a)). On Day 72, the removal in the non-inoculated filter, however, was still less efficient on a volumetric basis than the removal in the inoculated filter (i.e. approximately 30 cm required to reach compliance in the non-inoculated filter as opposed to 10 cm in the inoculated filter).

Manganese removal at the upper third of the inoculated filter was greatly increased by Day 35 (Figure 3(b)). At this time, nearly all manganese was removed at a depth of only 20 cm, meaning that the deeper layer of mature sand (20–60 cm) no longer contributed substantially to manganese removal. The top mature sand layer of the inoculated filter (0–10 cm) showed an increase in manganese removal over time (Figure 3(b)). The greatest manganese removal rate was observed at Day 72 in the inoculated filter from 0–10 cm depth. In this interval, the removal rate for an initial manganese concentration of 0.3 mg/L was approximately 0.2 mg/L/min. Similar manganese removal rates were reported by Dangeti *et al.*

(2017) after inoculation of a pilot-scale biofilter with back-wash sludge.

The accumulated amount of manganese removed at each depth of each filter was calculated based on the water samples from the manganese profiles (Figure 3(c)). Interestingly, the amount of manganese removed from the 10–20 cm layer in the inoculated filter (initially virgin sand) was similar to the amount removed from the 0–10 cm layer (initially mature sand). This suggests that manganese removal capacity was transferred from the mature sand to the virgin sand layer directly underneath. It should be noted that removed manganese at 0–10 and 10–20 cm layers diverged near the end of the experiment, not because the 0–10 cm layer was more efficient, but due to the 10–20 cm layer receiving lower manganese concentrations.

Manganese coating in inoculated and non-inoculated filters during the start-up period

The manganese coating on the initially virgin sand grains showed an increase over time, whereas the manganese coating on the mature sand at 10 cm depth of the inoculated filter remained in the order of 10 mg/g medium (Figure 4).

By Day 39, the manganese coating the grains at 20 cm depth of the inoculated filter was 1% of the manganese coating at 10 cm depth (Figure 4). Despite this difference, the manganese removal profile of the inoculated filter at Day 39 showed

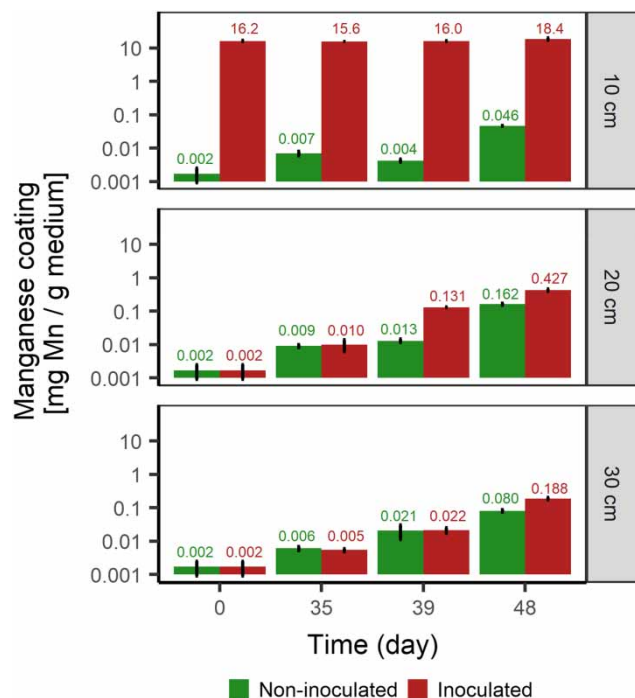


Figure 4 | Manganese coating development in duplicates at depth 10, 20 and 30 cm at Day 35, 39 and 48 from the non-inoculated filter (green) and from the inoculated filter (red). Numbers are the average of the replicates and error lines indicate standard deviation between replicates. Please refer to the online version of this paper to see this figure in colour: <http://dx.doi.org/10.2166/wqrj.2018.016>.

that comparable amounts of manganese were removed by those layers (Figure 3(b)). These results suggest that accumulation of approximately 1% (≈ 0.1 mg/g) of the manganese coating the matured filter sand was sufficient for the initially virgin sand to achieve a performance comparable to the fully matured sand. These results indicate that freshly precipitated manganese oxide on the initially virgin filter is more efficient than precipitates on the initially matured grains.

At Day 48, the amount of manganese coating on the initially virgin medium at 20 cm and 30 cm depth of the inoculated filter was twice as high as the non-inoculated filter (Figure 4). The same two-fold difference was observed when comparing the total manganese removed by the 10–20 cm layer of each filter by Day 48 (20 cm depth, Figure 3(c)).

Manganese removal with and without NaN_3 in batch and column assays

NaN_3 is an inhibitor of respiratory activity in microorganisms while it does not appear to affect autocatalytic

properties of MnOx coatings (Rosson *et al.* 1984). NaN_3 addition was used in the current study to compare manganese removal processes related to physico-chemical and biological mechanisms. Manganese removal observed in medium samples with NaN_3 was assumed to be mainly due to physico-chemical processes, and the difference between the manganese removal observed in medium samples with and without NaN_3 was assumed to be mainly due to biological processes (Figure 5). To identify the dominant process in manganese removal, the ratio between apparent physico-chemical and biological removal rates was calculated. When the ratio was <1 , most manganese removal was attributed to biological processes, and when the ratio was >1 most manganese removal was attributed to physico-chemical processes.

Medium samples collected from the non-inoculated filter during the start-up period of manganese removal indicated that the manganese removal was attained by both biological and physico-chemical processes (Day 35 and 39, Figure 5(a)). During that period, the ratio between physico-chemical and biological removal rates was 1 on average and showed no statistical difference over depth ($p > 0.05$ after Mann-Whitney test). In contrast, physico-chemical removal mechanisms appeared to dominate after the start-up period at the deeper biofilter layers (depth 20 and 30 cm) with an average ratio of 15, whereas biological mechanisms remained important for manganese removal at 10 cm depth of the filter with an average ratio of 0.5 (Day 48, Figure 5(a)).

The manganese removal rate of the non-inoculated filter at Day 48 due to physico-chemical processes was three times higher at 20 cm than at 10 cm depth (Figure 5(a)). Similarly, the amount of manganese coating on the grains of the non-inoculated filter at Day 48 was 3.5 times higher at 20 cm than at 10 cm depth (0.162 mg Mn/g medium and 0.046 mg Mn/g medium respectively, Figure 4).

In contrast to the non-inoculated filter, no time dependent change was observed in the manganese removal processes occurring in the inoculated filter. Filter medium samples from all depths showed that the manganese removal from Day 35 to Day 48 was mostly due to physico-chemical processes (Figure 5(b)). Further, the ratio between physico-chemical and biological removal rates showed no statistical difference over depth ($p > 0.05$ after Mann-Whitney test).

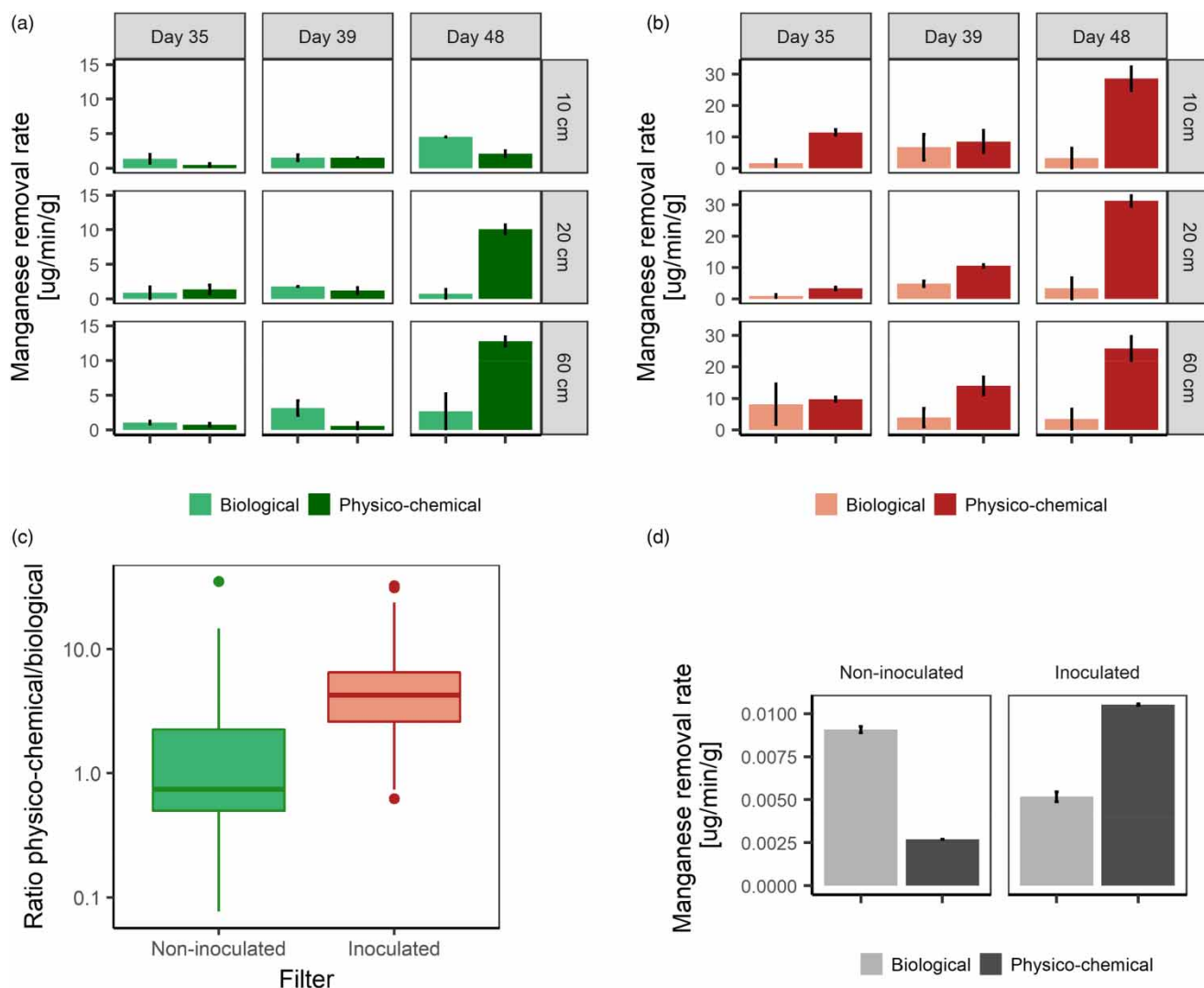


Figure 5 | Manganese removal rates in batch assay associated to biological and physico-chemical processes in filter medium samples in duplicates collected from depth 10, 20 and 60 cm on Day 35, 39 and 48 from (a) the non-inoculated filter and (b) the inoculated filter. (c) Boxplot of the ratio between physico-chemical and biological processes occurring at Day 35, 39 and 48 of both filters ($n = 18$). (d) Manganese removal rates in column assay associated to biological and physico-chemical processes in filter medium samples collected from 0–10 cm layer of both pilot filters at Day 72. Error lines indicate standard deviation between replicates.

The initially matured sand layers in the inoculated filter more than doubled their manganese removal rates over time from Day 35 to Day 48. This increase in manganese removal at 10 cm depth (initially matured sand) of the inoculated filter was also observed in the manganese profiles (Figure 3(b)). From Day 39, the initially virgin sand layer at 20 cm depth of the inoculated filter showed similar manganese removal rates as media samples from the initially matured sand layers (0–10 cm and 50–60 cm, Figure 5(b)). This similar performance in manganese removal at 10 cm and 20 cm depth (initially matured

sand and initially virgin sand, respectively) was also observed in the manganese profiles of the inoculated filter (Figure 3(b)).

Overall, the sum of the physico-chemical and biological manganese removal rates in media samples from the inoculated filter was on average two times higher than the total manganese removal rates from the non-inoculated filter (Figure 5(a) and 5(b)). A two-fold difference between the filters was also observed at 20 cm depth by Day 48 in the total manganese removal of the pilot filters (Figure 3(c)) and in the MnOx coating (Figure 4).

The ratio between physico-chemical and biological removal rates of all medium samples was statistically different between the filters ($p < 1 \times 10^{-11}$ after Mann-Whitney test), with a 0.8 median for the non-inoculated filter indicating a more active role of biological processes, and a median of 4.3 for the inoculated filter suggesting a more active role of physico-chemical processes (Figure 5(c)).

A column assay with and without NaN_3 addition was conducted using filter medium collected at Day 72 from the inoculated and non-inoculated filters at 10 cm depth (Figure 5(c)). The results indicated that the contribution of biological processes continued to be most pronounced on samples from the top 10 cm of the non-inoculated filter (Figure 5(c)). In total the manganese removal rates observed in each column were similar to the manganese removal rates from the top 10 cm of each filter at Day 72 ($0.009 \mu\text{g}/\text{min}/\text{g}$ medium in the non-inoculated filter and $0.014 \mu\text{g}/\text{min}/\text{g}$ medium in the inoculated filter).

Fully matured samples collected from the second stage filter of Fredensborg waterworks (Figure S1, supplementary material, available with the online version of this paper) showed a physico-chemical to biological manganese removal ratio of 5.6, indicating a limited contribution of biological means to manganese removal in matured biofilters (47 years old). This suggests that the contribution of biological mechanisms in the removal of manganese continues to be less prominent in fully matured biofilters compared to filters during start-up. Bruins (2016) showed a clear difference in structure between biologically and physico-chemically formed MnOx (Birnessite) in matured filters. Currently, the importance that biogenic manganese oxides might have in the long-term efficiency of manganese removal in matured biofilters is not known.

In related work, Vandenabeele *et al.* (1992) investigated the manganese removal capacity of matured sand (for decades removing manganese) in a PYM-medium (using peptone, yeast extract and manganese sulfate) with and without addition of NaN_3 (15 mM). The results showed that addition of NaN_3 reduced the manganese removal by 50% after 5 days of incubation but had no effect on the manganese removal under 24 h of incubation. In another study, Olańczuk-Neyman & Bray (2000) investigated the role of physico-chemical and biological processes in manganese removal from groundwater using NaN_3 (15 mM) on

matured sand (no reference to maturation age but reported to remove manganese successfully). The results suggested that the removal of manganese on matured sand occurred mostly due to autocatalytic oxidation by the previously formed manganese oxides (by physico-chemical and biological processes). In a more recent work, Sahabi *et al.* (2009) investigated biotic and abiotic manganese removal in fully matured filter medium samples with and without NaN_3 (10 mM). The results showed that biological processes had a 50% contribution to manganese removal in a 3-year-old matured anthracite medium but no significant role in a 15-year-old mature anthracite medium (Sahabi *et al.* 2009). Hence, the results obtained in the current study complement previous investigations of matured filters, suggesting that the physico-chemical processes in manganese removal increase with filter medium age.

Bacterial diversity in inoculated and non-inoculated filters during the start-up period

Despite reducing the duration of the start-up period and enhancing the manganese removal capacity of the filter (Figures 2 and 5), the effect of initially matured sand layers on the microbial community formed on initially virgin sand layers is unknown for this inoculation method. To better understand this, source water samples and filter medium samples collected from several depths of both pilot-scale filters were analyzed by 16S rDNA amplicon sequencing.

The relative abundance of the top 20 most abundant bacterial genera of all samples is included in the supplementary material (available online). A PCA based on all taxa detected in each sample identifies two main clustering areas: initially virgin sand and initially matured sand (Figure 6). The microbial community of medium samples of initially virgin sand showed changes in diversity over time, e.g. three medium samples of initially virgin sand collected from the non-inoculated filter at Day 35 and 39 showed a closer proximity to the microbial diversity of the source water (Figure 6). In contrast, the microbial diversity on initially matured sand samples showed a clear clustering over time that was distinct when compared to the bacterial communities developed on the initially virgin sand. Overall, the microbial community of the medium samples clustered according to the filter medium

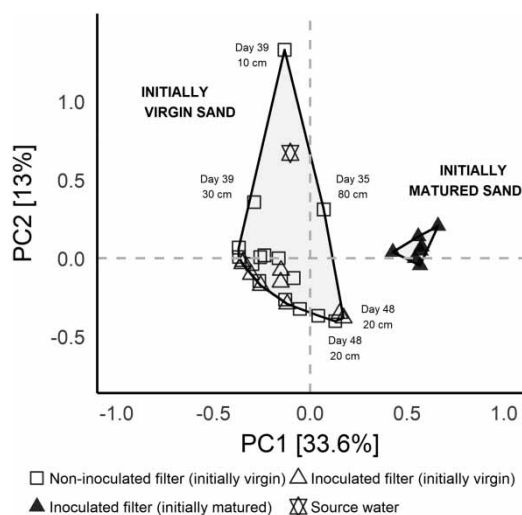


Figure 6 | PCA of bacterial community present in the source water and in filter medium samples collected at depth 10, 20, 30, 60 and 80 cm from both filters at Day 0, 35, 39 and 48. Each point represents the bacterial community present in the source water and in the medium samples collected from the non-inoculated filter and the inoculated filter.

type (initially virgin or matured), suggesting that the initially matured sand layers located at depth 10 and 60 cm of the inoculated filter had limited effect on the microbial community developed on initially virgin sand layers during the 48 days of the experiment (Figure 6). Hence, significantly longer time is likely required to obtain fully matured microbial communities.

Bai et al. (2016) investigated the microbial diversity after start-up of bioaugmented and non-bioaugmented columns removing Mn(II), Fe(II), As(III) and Sb(III) using a manganese oxidizing bacterium (*Pseudomonas* sp. QJX-1). Results showed higher overall treatment efficiency by the bioaugmented columns but no significant difference between the bacterial community of bioaugmented and non-bioaugmented columns after 120 days. Our results complement the previous ones by indicating limited immediate effect of inoculation on the microbial community when using a microbial consortium inoculation method with addition of matured sand.

CONCLUSIONS

Manganese removal and microbial community development of a non-coated virgin sand pilot filter with and without

inoculation with 20% matured sand was monitored for a period of 72 days. Based on findings in this study, the following is concluded:

- The non-inoculated filter took 35 days to initiate significant manganese removal and 41 days to comply with manganese water quality criteria. The inoculated filter showed significant initial removal from Day 0 and compliance from Day 25.
- During the first 48 days of operations, similar amounts of manganese were removed in the inoculated filter by the top layer of matured sand and the following layer of virgin sand. By Day 48, the inoculated filter showed two times higher manganese removal rates and manganese in the coating of filter media.
- From the onset of manganese removal to compliance, both physico-chemical and biological processes contributed to the manganese removal in the non-inoculated filter. One week after compliance, biological mechanisms remained important for manganese removal at the top 10 cm of the non-inoculated filter, whereas physico-chemical processes were of more importance at deeper filter layers.
- The major manganese removal processes occurring in the filters were statistically different. The non-inoculated filter was dominated by biological processes, whereas physico-chemical processes were of more importance in the inoculated filter. Inoculation appeared to mainly enhance the physico-chemical manganese removal potential during start-up.
- The use of proactive inoculation by addition of matured filter sand contributes to a shorter start-up period of biofilters with limited effect on the microbial community developed in the adjacent layers of the filter.

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